PRELIMINARY AND SHORT REPORTS

EFFECT OF THALLIUM ON SULFHYDRYL COMPOUNDS IN VITRO*

PETER FLESCH, M.D., PH.D. AND SHELDON B. GOLDSTONE, M.D.

In spite of the large amount of experimental work done with thallium (1), its mode of action in producing alopecia has not been fully elucidated. Gross *et al.* (2) postulated that thallium, like many other heavy metals, may react with the free sulfhydryl groups of cysteine and homocysteine. In this way cystine deficiency results and normal hair growth is inhibited. This assumption is not supported by toxicologic studies showing that rabbits (3) and rats (4) cannot be protected against thallium poisoning by administration of BAL. No data could be found in the literature regarding the action of thallium on tissues *in vitro*. It seemed therefore of interest to study the *in vitro* effect of thallium acetate upon sulfhydryl compounds and a sulfhydryl enzyme, succinic dehydrogenase.

EXPERIMENTAL

In order to ascertain whether thallium would bind free sulfhydryl groups, 0.001 to 0.1 M thallium acetate was added to freshly prepared glutathione solutions, containing 40 to 80 μg glutathione/ml.; after 15 minutes incubation at room temperature or in a water bath at 37°C., determinations of free sulfhydryl groups were carried out with Anson's method (5). The same procedure was used to determine the effect of thallium acetate upon the free sulfhydryl groups of 1% aqueous mouse liver homogenates.

The effect of thallium acetate upon succinic dehydrogenase activity was measured with the colorimetric method of Kun and Abood (6) in mouse liver and mouse skin homogenates. For each determination four buffered (pH 7.4) samples of 10% aqueous liver homogenate were allowed to stand at room temperature or in a water bath of 37°C. for 15 minutes. To one of the samples 0.2 M sodium succinate was mixed with 0.001 to 0.1 M thallium acetate; the second contained thallium acetate, but no succinate; to the third sample succinate was added, but no thallium acetate; the fourth had liver homogenate only. All four tubes were then incubated in a water bath of 37°C. for 15 or 30 minutes. The difference in colorimeter readings between the samples containing the substrate succinate gave the degree of inhibition of succinic dehydrogenase. The other two samples without the substrate served as control blanks.

The same experiment was carried out on mouse skin homogenates, prepared by chilling and thoroughly mincing the skins of four mice. Care was taken to separate the skin completely from the underlying muscle. The skins were then homogenized with glass homogenizers in an ice bath to the consistency of a thick paste with 6 ml. distilled water. Because of the impossibility to obtain complete homogenization with disruption of epidermal cells in the mouse skin with this method, incubation of the skin homogenates with the substrate was prolonged for one hour at 37°C.

RESULTS

There was no inactivation of free sulfhydryl groups when 0.1 to 0.2 ml. thallium acetate was added in concentrations up to 0.1 M to 60–100 μg glutathione or to 10 mg. homogenized mouse liver. When the concentration of thallium acetate was increased, a small (up to 20%)

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* From the Department of Dermatology and Syphilology, School of Medicine (Dr. Donald M. Pillsbury, Director), University of Pennsylvania, Philadelphia.

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inhibition was observed. Similarly, in concentrations less than 0.01 M, thallium acetate had no effect upon the succinic dehydrogenase activity of mouse liver homogenates. In higher concentrations, thallium acetate caused partial inactivation of succinic dehydrogenase. The inhibition of the enzyme activity gave a linear relationship when plotted against the concentration of thallium acetate on semilogarithmic paper (Fig. 1). Inhibition of succinic dehydrogenase activity of mouse liver could not be reversed by the addition of glutathione (100 μg/100 mg. tissue) or of 0.1 M sodium thioglycollate.

A small, but consistent succinic dehydrogenase activity was observed in mouse skin homogenates. Inhibition of this enzyme activity by thallium acetate was in the same range as that found for liver homogenates.

DISCUSSION

These experiments indicate that the depilatory effect of thallium is not due to a direct interference with normal sulfhydryl metabolism. It must be emphasized that the inactivation of free sulfhydryl groups, as well as the inhibition of succinic dehydrogenase activity

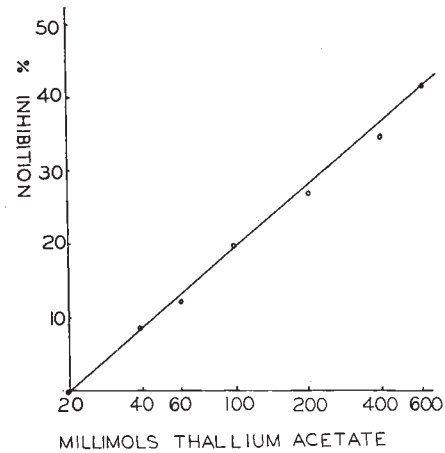

FIG. 1. Inhibition of succinic dehydrogenase activity of mouse liver by thallium acetate.

occurred with concentrations of thallium acetate (21–26 mg./g. tissue) which are over a thousand times higher than tissue concentrations of thallium reported in cases of fatal poisoning (15 μg/g. tissue) (7). Unless it is assumed that there is a considerable accumulation of thallium in the skin—an assumption, not supported by experimental data (8)—it is unlikely that hair loss occurring in thallium poisoning is caused by a direct interference with sulfhydryl metabolism of the skin. The theory that thallium may interfere with the synthesis or utilization of cystine (4) is not contradicted by these experiments.

No previous data were found in the literature concerning succinic dehydrogenase activity in skin. Demonstration of this enzyme in skin supports the pathways of cutaneous carbohydrate metabolism postulated by Barron *et al.* (9).

The failure of thallium to inhibit free sulfhydryl groups *in vitro* is in agreement with toxicologic findings indicating that animals cannot be protected against thallium poisoning by administration of BAL (3, 4). The claimed beneficial effect of BAL in the treatment of subacute thallium poisoning in man (10) has at present no rational experimental basis.

SUMMARY

1. Thallium acetate in 0.001 to 0.01 M concentrations had no effect upon free sulfhydryl groups of glutathione and mouse liver homogenates. Higher concentrations resulted in slight inhibition.

2. Succinic dehydrogenase activity in mouse skin and liver was inhibited by thallium acetate in 0.1 M and higher concentrations. These concentrations are far above those occurring in tissues of animals poisoned with thallium.

3. Small, but consistent succinic dehydrogenase activity was observed in mouse skin homogenates.

4. Treatment of thallium poisoning with BAL is at present without experimental foundation.

ADDENDUM

After completion of the manuscript we came across a paper by Goffart (Arch. Intern. Pharmacodynamie, **74:** 9, 1947). This author incubated human skin with various sulfhydryl inhibitors and then determined the epidermal sulfhydryl by a histochemical method (nitroprusside stain). He found that even after three hours' incubation thallium acetate in a 1% concentration had no effect on the free sulfhydryl groups of human skin. More prolonged incubation resulted in slight inhibition only.

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